TOXOPLASMOSIS IN SINGAPORE

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Abstract. The present paper reports research done in the following areas in the Parasitology Unit of the Department of Microbiology, National University of Singapore: determination of anti-*Toxoplasma gondii* antibodies in different IgG subclasses; immunoblot analysis of the antibody response to *T. gondii* and identification of key reactive epitopes; production and characterization of monoclonal antibodies to *T. gondii*; and the development of an IgM capture ELISA.

IgG1 is the dominant subclass involved in the humoral response to *T. gondii*. IgG3 and IgG4 may be produced at low but significant levels. IgG2 production was not apparent. Immunoblot analysis of the positive sera revealed that the major antigens recognized by IgG and IgM antibodies were of MW 22, 35 and 67 kDa. Many of the bands observed in blots with positive sera were also present in blots with negative sera. The 22 kDa band was seen in majority of positive sera and was generally absent in negative sera. Anti-*Toxoplasma* sera also revealed IgA antibodies. Immunoblot analysis of the antigen revealed a 14 kDa band that reacted with all IgA positive sera. From a panel of monoclonals produced against *T. gondii*, one monoclonal, 5F3 (A) was selected for development of a monoclonal based IgM capture ELISA for detection of antibodies to 22 kDa and 41 kDa epitopes of *T. gondii*.

INTRODUCTION

Seroprevalence studies have shown that human toxoplasmosis is important in Singapore as reviewed by Singh (1990). Cases of lymphadenopathy, ocular disease and congenital infections have been reported from various hospitals and clinics.

Currently, several hospitals and private laboratories conduct routine diagnosis of toxoplasmosis by serology. All of them use commercially available tests. The test of choice now is the ELISA for IgG and IgM determination although a few laboratories use the IFA test for routine diagnosis without discriminating the class of immunoglobulin involved (Singh and Chan, 1988). There is an urgent need for quality control with respect to *Toxoplasma* serology. Commercial considerations seem to outweigh scientific value with regard to the tests used by the laboratories. Interpretation of results from the different laboratories poses a problem to the clinicians.

Very few laboratories in Singapore indulge in research on toxoplasmosis. The Parasitology Unit of the Department of Microbiology at the National University of Singapore has been engaged in various areas of research on toxoplasmosis since the 1960s. The earlier studies helped to establish the base line data for the prevalence of this infection in Singapore in humans and animals. Over the last few years the research work has been into the serodiagnosis, antigenic analysis and production of monoclonal antibodies to *Toxoplasma gondii*. Some of this work is highlighted in this presentation.

Determination of anti-*Toxoplasma gondii* antibodies in different IgG subclasses

A study was undertaken (Ee *et al*, 1989) to determine the predominant subclasses in the IgG response to toxoplasmosis. The antibody activity of the four IgG subclasses in toxoplasmosis was determined by the indirect ELISA employing commercially available monoclonal antibodies to the four subclasses.

Forty-four sera positive by IgG-ELISA and 73 negative sera were tested for IgG subclasss activity. This study showed that IgG1 is the dominant IgG subclass involved in the humoral reponse to *T. gondii* infection in humans. IgG3 and IgG4 may be produced at low but significant levels while IgG2 production was not apparent. The results may reflect the preferential recognition of protein antigens of *T. gondii* by IgG1, IgG3 and to a smaller extent IgG4 antibodies.

In humans, IgG1 and IgG3 avidly bind complement and classical complement pathway activation has been demonstrated as the activation system for antibodies to *T. gondii* (Schreiber and Feldman, 1980). IgG1 and IgG3 also bind to mononuclear cells (Alexander, 1980) and activated macrophages and monocytes have been implicated as the major effector cell population in cell-mediated immunity to *T. gondii* (Wilson and Remington, 1979; McLeod *et al*, 1980). Phagocystis is promoted when cells bind with opsonized micro-organisms through receptors for IgG (IgG1 and IgG3) and complement (C3b). The possible role of IgG4 is unclear. It has been suggested that IgG4 antibodies become important when antigenic exposure is chronic (Aalberse *et al*, 1983).

Immunoblot analysis of human sera

Sera from healthy individuals and patients clinically suspected of toxoplasmosis were tested for IgG and IgM antibodies to *T. gondii* by latex agglutination and ELISA respectively. These sera were further analyzed for reactive antigens of *T. gondii* by immunoblotting.

IgG antibodies reacted with many antigens over a wide range of MW (16–110 kDa) while IgM antibodies recognized fewer antigens (22–110 kDa). The major antigens recognized by IgG and IgM antibodies were of MW 22, 35 and 67 kDa. Many of the bands observed in the blots of positive sera were also present on blots of negative sera. These bands were especially prominent in IgM blots. The 22 kDa antigen is a major component of the tachyzoite antigens (Kasper *et al*, 1982; Sharma *et al*, 1983; Kasper, 1987) and is recognized by both IgG and IgM antibodies. A 25 kDa antigen reported by Partanen *et al* (1984) may be similar to the 20–25 kDa antigen described by Potasman *et al* (1986). The 22 kDa antigen is seldom seen in blots of negative sera. Araujo *et al* (1984) showed that while antibodies cross-reacted with many antigens of *T. gondii* and *Hammondia hammondi*, a 21.5 kDa antigen appeared specific for *T. gondii*.

The IgA response to *T. gondii* was also analyzed by the indirect ELISA as well as by immunoblotting. IgA has been reported previously to be a useful marker in diagnosis of toxoplasmosis (Decoster *et al*, 1988). Sera positive for IgA antibodies showed bands of molecular weight 14–90 kDa. Faint bands of high MW (> 40 kDa) were sometimes present on blots of IgA negative sera. An antigen of MW 14 kDa appeared as a distinct band in all blots of IgA positive sera; it was absent in IgA negative sera. This finding contrasts with our earlier results of IgG and IgM blots where bands of MW <20 kDa were rarely seen; if present, they were rather faint and diffused. Studies of Handman *et al* (1980) and Araujo and Remington (1984) had shown that a 14 kDa antigen reacted strongly with IgG and IgM antibodies. The 14 kDa antigen had also been shown to be protective in mice, drastically reducing the mortality rate of infected mice (Araujo and Remington, 1984). IgA antibodies may play an important role in the early and accurate diagnosis of toxoplasmosis and the usefulness of the 14 kDa antigen needs to be investigated.

Monoclonal antibodies to *Toxoplasma gondii*

From fusions involving various immunization protocols, a panel of nine stable hybridomas secreting anti-*Toxoplasma* monoclonal antibodies (McAb) were selected for further studies. Isotyping tests showed that all the hybrids derived from fusions involving the *in vivo* immunization route produced antibodies of the IgG class but a single hybrid from the *in vitro* route produced IgM antibodies.

Localization of reactive antigens was determined by immunofluorescence assay and immunogold labelling. In the IFA test, most of the antibodies reacted with live tachyzoites indicating that these antibodies detected antigens which are present on the surface of the parasites. With two monoclonals, 2E5 and 3F2, reaction was more

intense with formalin-fixed parasites. Immunogold labeling studies were carried out with McAb 5F3(A) and 5F3 (B) only. With McAb 5F3 (A), gold-labelling was observed on the surface of the parasite; the density of the gold particles was however, rather low. McAb 5F3 (B) reacted only when the organisms were extracted with 0.002% saponin. Labelling was also observed on the surface.

The sensitivity of reactive *T. gondii* antigens to heat, pronase or periodate was investigated by screening for binding reactions between antibodies and antigens after the latter had been subjected to the various treatments. None of the antigens appeared to be sensitive to periodate oxidation.

The molecular weights of the antigens reacting with the monoclonals antibodies were determined by immunoblotting studies. The blotting patterns obtained showed that most of the antibodies reacted with more than one determinant.

Two monoclonals antibodies, 1G7 and 5F3 (A), detected both a 41 and 22 kDa antigen. Localization tests indicated that these antigens are found on the surface membrane of the parasite and are likely to be the P43 and P22 antigens described by the other laboratories. Under nonreducing conditions, the 22 kDa protein moved as a 18 kDa molecule which was also observed for P22 by Huskinson *et al* (1989). The similarity of reactions obtained with the IFA, the various physiochemical tests, and immunoblots suggest that the antigens recognized by the two differently derived antibodies 1G7 and 5F3(A) are probably the same. Both antibodies may recognize the same epitope or possibly different epitopes on each protein. The epitopes are sensitive to heat but not to pronase nor periodate.

In our studies, P22 was frequently detected in positive blots, and the prevalence in blots incubated with negative sera was low (unpublished data). This makes P22 an attractive candidate antigen for diagnostic tests. Monoclonal antibody 5F3(A) recognized both a 41 and 22 kDa antigen. Characterization tests indicated that these were located on the surface membrane of the tachyzoites and were likely to correspond to the major surface proteins with similar MW described in other studies (Handman *et al*, 1980; Kasper *et al*, 1983; Courveur *et al*, 1988). It is not a disadvantage to use a monoclonal antibody that detects in a specific manner, two rather than one major parasite antigen.

A monoclonal-based IgM capture ELISA for detection of antibodies to 22 kDa and 41 kDa epitopes of *Toxoplasma gondii*

The murine monoclonal antibody, 5F3(A) which reacts with a 22 and 41 kDa *T. gondii* surface antigen was employed in an IgM capture ELISA. A total of 125 sera were analyzed by both a commercial ELISA (polyclonal-based Abbott Toxo-M EIA) and the monoclonal-based IgM capture ELISA. The ELISA values were compared with the Abbott Toxo-M Index. A good coefficient of corrrelation of 0.91 ($p < 0.01$) was obtained when results of sera tested by both these systems were compared.

To conclude, we have successfully devised a specific anti-*Toxoplasma* IgM capture ELISA using a monoclonal antibody that detects both 41 and 22 kDa parasite antigens. The ELISA test established is simple, sensitive, and specific and may be used in the diagnosis of current *Toxoplasma* infection. Improvements that may be made include the conjugation of the horseradish peroxidase enzyme to the monoclonal antibody. This would eliminate one incubation step, thus providing for a faster assay.

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